

## CLAIMS

What is claimed is:

- 1                   1.     A primer set comprising:  
2                   (a)     at least two primers capable of amplifying a portion of all  
3 human leukocyte antigen (HLA) alleles of an HLA locus; and  
4                   (b)     a control primer pair capable of producing an HLA control  
5 amplicon of predetermined size by amplifying a portion of a HLA allele only if the  
6 HLA locus is present in a sample.
- 1                   2.     The primer set of claim 1 wherein the portion of the HLA allele  
2 amplified by the control primer pair is common to all or substantially all HLA alleles.
- 1                   3.     The primer set of claim 1 wherein the portion of the HLA allele  
2 amplified by the control primer pair comprises a portion of exon 4 of the HLA A  
3 locus or exon 4 of the HLA B locus.
- 1                   4.     The primer set of claim 1 wherein the predetermined size of the  
2 HLA control amplicon is about 500 to 1000 base pairs in length.
- 1                   5.     The primer set of claim 1 wherein at least one of the at least  
2 two primers has a 5' portion that is not complementary to the HLA allele.
- 1                   6.     The primer set of claim 5 wherein the 5' non-complementary  
2 portion decreases a melting temperature (T<sub>m</sub>) between the primer and a HLA allele,  
3 further wherein the decreased melting temperature results in an enhanced specificity  
4 of an amplification reaction.
- 1                   7.     The primer set of claim 5 wherein the 5' non-complementary  
2 portion allows for amplification of a more abundant product, further wherein the 5'  
3 portion allows for a more robust amplification reaction.

- 1                   8.     A primer set comprising:  
2                   (a)     a multiplicity of primers capable of simultaneously amplifying  
3 a plurality of a portion of Class I HLA alleles of a HLA locus under a single set of  
4 reaction conditions in a multiplex polymerase chain reaction.
- 1                   9.     The primer set of claim 8 wherein the plurality of a portion of  
2 Class I HLA alleles belong to a same HLA locus.
- 1                   10.    The primer set of claim 6 wherein the same HLA locus is a  
2 HLA A or a HLA B locus.
- 1                   11.    The primer set of claim 5 wherein the multiplicity of primers  
2 are capable of producing a first amplicon and a second amplicon from the HLA locus.
- 1                   12.    The primer set of claim 8 wherein the first amplicon spans exon  
2 1 to intron 3 and the second amplicon spans intron 3 to exon 5.
- 1                   13.    The primer set of claim 8 wherein at least one of the  
2 multiplicity of primers has a 5' portion that is not complementary to the portion of the  
3 Class I HLA allele.
- 1                   14.    The primer set of claim 13 wherein the 5' non-complementary  
2 portion allows a decrease in a melting temperature ( $T_m$ ) between the primer and a  
3 HLA allele, further wherein the decreased melting temperature results in an enhanced  
4 specificity of an amplification reaction.
- 1                   15.    The primer set of claim 13 wherein the 5' non-complementary  
2 portion allows a more abundant product during amplification, further wherein the 5'  
3 portion allows a more robust amplification reaction.
- 1                   16.    A primer for sequencing an HLA allele comprising:  
2                   (a)     a primer comprising a 3' portion and a 5' portion wherein the 3'  
3 portion is complementary to an HLA allele and the 5' portion is not complementary to  
4 the HLA allele, wherein the primer allows complete resolution of an exonic sequence  
5 by a sequencing reaction.

1                    17.    The primer of claim 16 wherein the 5' non-complementary  
2    portion is 1 to about 35 bases.

1                    18.    The primer of claim 16 wherein the primer allows complete  
2    resolution for one of exon 2 or exon 3 in an allele of the HLA B locus.

1                    19.    The primer of claim 16 wherein the primer allows complete  
2    resolution of exon 1 in an allele of the HLA B locus.

1                    20.    The primer of claim 16 further comprising at least one  
2    additional primer complementary to a different HLA allele.

1                    21.    The primer of claim 16 wherein the 5' non-complementary  
2    portion allows a single electrophoresis gel to be used for all sequencing products.

1                    22.    The primer set of claim 16 wherein the 5' non-complementary  
2    portion allows a decrease in a melting temperature ( $T_m$ ) between the primer and a  
3    HLA allele, further wherein the decreased melting temperature results in an enhanced  
4    specificity of a sequencing reaction.

1                    23.    The primer set of claim 16 wherein the 5' non-complementary  
2    portion allows a more abundant product during sequencing, further wherein the 5'  
3    portion allows a more robust sequencing reaction.

1                    24.    A primer set comprising:  
2                    (a)    a multiplicity of primers capable of simultaneously sequencing  
3    a plurality of HLA alleles of a HLA locus under a single set of reaction conditions in  
4    a multiplex sequencing reaction.

1                    25.    The primer set of claim 24 wherein the plurality of HLA alleles  
2    is a plurality of a portion of HLA alleles.

1                    26.    The primer set of claim 24 wherein the HLA locus comprises  
2    all loci of HLA Class I.

1                   27.     The primer set of claim 24 wherein the HLA locus comprises  
2 all loci of HLA Class II.

1                   28.     The primer set of claim 24 wherein the HLA locus comprises  
2 all loci of DRB.

1                   29.     A method for amplifying a class I HLA allele comprising:  
2                   (a)     performing an amplification reaction on a sample having or  
3 suspected of having a Class I HLA allele wherein the amplification reaction utilizes  
4 the primer set of claim 8.

1                   30.     The method of claim 29 further comprising sequencing any  
2 resulting HLA amplicons.

1                   31.     The method of claim 29 wherein the sample is a cDNA.

1                   32.     A method for detecting the presence of an HLA allele  
2 comprising:  
3                   (a)     amplifying a nucleic acid wherein the amplification reaction  
4 comprises at least two primers capable of amplifying all HLA alleles of an HLA locus  
5 and a control primer pair capable of producing an HLA control amplicon of  
6 predetermined by amplifying a portion of a HLA allele only if the HLA locus is  
7 present in the sample; and  
8                   (b)     detecting the presence of the HLA allele.

1                   33.     The method of claim 32 wherein the portion of the HLA allele  
2 amplified by the control primer pair is common to all or substantially all HLA alleles.

1                   34.     The method of claim 33 wherein the portion of the HLA allele  
2 amplified by the control primer pair comprises a portion of exon 4 of the HLA A  
3 locus or exon 4 of the HLA B locus.

1                   35.     The method of claim 32 wherein predetermined size of the  
2 HLA control amplicon is about 500 to 2200 base pairs in length.

1                   36.     The method of claim 32 wherein the nucleic acid is a cDNA.

1                   37.     The method of claim 32 wherein detecting the presence of the  
2 HLA allele comprises whole HLA locus sequencing.

1                   38.     The method of claim 32 wherein detecting the presence of the  
2 HLA allele comprises partial HLA locus sequencing.

1                   39.     A method for isolating and amplifying an HLA allele comprising:  
2                   (a)     reverse transcribing a RNA from a sample to form a cDNA; and  
3                   (b)     performing an amplification reaction on the cDNA, wherein the  
4 amplification reaction utilizes the primer set of claim 8.

1                   40.     The method of claim 39 further comprising performing step (a)  
2 and step (b) simultaneously.

1                   41.     A method for amplifying and detecting the presence of an HLA  
2 allele comprising:

3                   (a)     amplifying a nucleic acid wherein the amplification reaction  
4 comprises at least three primers capable of amplifying all HLA alleles of an HLA  
5 locus in a multiplex amplification reaction; and

6                   (b)     detecting the presence of the HLA allele.

1                   42.     The method of claim 41 wherein detecting the presence of the  
2 HLA allele comprises sequencing the amplified nucleic acid in a multiplex  
3 sequencing reaction.

1                   43.     The method of claim 41 wherein step (a) and step (b) are  
2 automated.

1                   44.     The method of claim 43 further comprising automation on an  
2 array.

- 1                   45.     A kit for amplifying and detecting human leukocyte antigen  
2 alleles comprising:
- 3                   (a)     at least two primers capable of amplifying a portion of all  
4 human leukocyte antigen (HLA) alleles of an HLA locus; and a control primer pair  
5 capable of producing an HLA control amplicon of predetermined size by amplifying a  
6 portion of a HLA allele only if the HLA locus is present in a sample; and
- 7                   (b)     at least one primer comprising a 3' portion and a 5' portion  
8 wherein the 3' portion is complementary to an HLA allele and the 5' portion is not  
9 complementary to the HLA allele, wherein the primer allows complete resolution of  
10 an exonic sequence by a sequencing reaction.